

What is claimed is:

1. A method for chemical modification of human Interleukin-3, preferably for the introduction of one or more of the following features: enhanced biological activity, enhanced stability, suppressed antigenicity, acquired antagonistic activity or cell inhibitory activity.
2. A method according to claim 1, wherein the modification is a gradual modification, preferably under gradual varying conditions, wherein one or more of the following conditions are varied: pH between 5.0 and 7.0, preferably in steps of 0.5 pH units, and/or time or reagent-concentrations.
3. A method according to one or more of the preceding claims, wherein the substrate is not human IL-3 but one or more of the following preferably human proteins or peptides: Other Interleukins, hemopoietic growth factors, peptide hormones or protein hormones, signal peptides or signal proteins, biologically active proteins or peptides.
4. A method according to one or more of the preceding claims, wherein the antigenicity is lowered by shielding possible interactions of antigenic response inducing amino acids in the protein or peptide.
5. A method according to one or more of the preceding claims, wherein the stability is changed, preferably because of shielding possible interactions of amino acids that form a binding place for protease's.
6. A method according to one or more of the preceding claims, wherein the receptor binding of the peptide or protein is enhanced by shielding the residues that reduce this receptor binding.
7. A method according to one or more of the preceding claims, wherein the receptor binding of the peptide or protein is enhanced by the introduction of a new chemical interaction, preferably a charge, preferably a negative charge.
8. A method according to one or more of the preceding claims, wherein the modification is specific for a few types of amino acid, one type of amino acid, for instance amine-residues and/or even 1 amine-residue in the peptide or protein, for instance the N- terminus.
9. A method according to one or more of the preceding claims, wherein the modification has specificity to one or more residues that are involved in catalytic activity, preferably His-residues.
10. A method according to one or more of the preceding claims, wherein the modification has specificity to one or more residues that are involved in catalytic activity, preferably His-residues for the introduction of an antagonistic and/or cell inhibitory activity.

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11. A method for the chemical or non chemical modification of proteins and peptides for the introduction of an antagonistic and/or cell inhibitory activity by disruption of phosphate binding.
12. A method for specific chemical modification of selected amino acids on a peptide or protein using gradual chemical modification and reversible reagents.
13. A method for localizing chemically modified amino acids by native electrophoresis to determine change in charge, protease treatment and mass spectrometry, preferably laser desorption mass spectrometry.
14. A method for localizing biologically important residues on a protein or peptide, by chemical modification, preferably in a gradual manner, native electrophoresis, activity tests and localization of modified residues as described in previous claims.
15. A method for gradual chemical modification of biologically active proteins or peptides as described in one or more of the preceding claims, wherein the modification can be performed in a very specific manner by using previously described methods for localizing residues on a protein or peptide that are involved in biological activity.
16. Human Interleukin-3, modified only at one or more of the following residues: Ala¹, His²⁶, Lys²⁸, Lys⁶⁶, His⁹⁵, His⁹⁸, Lys¹⁰⁰, or Lys¹¹⁶.
17. Any preparation, containing a modified peptide or protein (both in mixed form and in chemically bound form) that is prepared according to one or more of the preceding claims.
18. A modified signal substance, preferably a protein hormone, peptide hormone, a growth factor, a hemopoietic growth factor, an Interferon, an interleukin and/or a colony stimulating factor wherein the modification is within or in close proximity to a partial or complete catalytic center.
19. A substance, as described in one or more of the preceding claims, wherein the catalytic activity is changed.
20. A substance, as described in one or more of the preceding claims, wherein the modification is within or in close proximity to a metal binding center, preferably a Zinc binding center.
21. A substance, as described in one or more of the preceding claims, wherein the metal ion is within or in close proximity to a catalytic center.
22. A substance, as described in one or more of the preceding claims, wherein the metal ion has a catalytic function in the unmodified substance.
23. A substance, as described in one or more of the preceding claims, wherein the metal binding properties have been changed.

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24. A substance, as described in one or more of the preceding claims, wherein the affinity of the signal substance for the receptor has not decreased for more than a factor 10, has remained the same or has even been increased.
25. A substance, as described in one or more of the preceding claims, wherein an enhanced biological activity, antagonistic activity and/or cell inhibitory activity has been obtained.
26. A substance, as described in one or more of the preceding claims, wherein the modification is a modification of an amino acid. This can be a chemical modification, preferably an alkylation and/or an acylation or molecular biological modification like a deletion mutation and/or a substitution mutation.
27. A substance, as described in one or more of the preceding claims, wherein the modified amino acid is involved in the binding of a metal ion, preferably a Histidine residue.
28. A substance, as described in one or more of the preceding claims, wherein the signal peptide is a Zinc binding signal peptide, preferably one or more of the following: IL-2, IL-3, IL-6, IFN-gamma, Growth Hormone, Prolactin and/or Insulin.
29. A substance, as described in one or more of the preceding claims, wherein the signal peptide is a growth factor with receptors from the same (cytokine) superfamily as the IL-3 receptor, preferably IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF and/or Epo.
30. A substance, as described in one or more of the preceding claims, wherein the substance has acquired a change in stability, preferably an enhanced stability.
31. A substance, as described in one or more of the preceding claims, wherein the substance has acquired a lowered stability, preferably in combination with an antagonistic activity.
32. DNA-constructs that contain the genetic code for the proteins and/or peptides as described in 1 or more of the preceding claims.
33. Any preparation containing one or more substances, (both in mixed form and in chemically bound form), that is described in one or more of the preceding claims or is prepared according to one or more of the preceding claims.
34. The use of any preparation as described in one or more of the preceding claims.
35. The use of any preparation as described in one or more of the preceding claims, preferably for one or more applications as described in the field of applications in this patent-description.

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36. Inhibition, suppression and/or the cure of a HIV infection by suppression of antibody production by B-cells and/or the suppression of generation and/or maturation of B-cells, preferably by a preparation as described by one or more of the preceding claims.
37. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein the antibody levels are lowered, preferably by plasmaphoresis, partial or complete plasma recovery or selective return of the serum.
38. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein the selection is performed *in vitro* , preferably by removal of antibodies, preferably HIV-reactive antibodies, preferably HIV-envelope reactive antibodies.
39. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein leukophoresis is performed.
40. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein it is achieved to lower the number of B-cells, preferably anti-HIV- antibody producing B-cells, preferably anti-HIV coat-antibody producing B-cells.
41. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein *in vivo* depletion is included, preferably with antibodies, preferably against HIV, preferably against the HIV envelope.
43. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein *in vivo* depletion of antibodies is achieved for instance by other antibodies.
44. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein there is a use of bi-specific antibodies, preferably directed against the combination CD19/CD3 and or CD20/CD3.
45. A method and or product as described in more of the preceding claims, wherein there is a use of B-cell apoptose induci substances, preferably APO-1.
46. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein there is use of an other inhibition of B-cell antibody production preferably by TGF-beta.
47. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein activation of provirus of the HIV infected subject is performed, preferably by administration of growth factors, preferably cytokines, preferably IL-2.

48. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein passive immune therapy is included, preferably with immune globulin of HIV-uninfected subjects.
49. A method and/or a product as described in- , or in combination with-, one or more of the preceding claims, wherein there is a use of a metal ion, preferably Zinc, to obtain one or more of the effects and/or results and/or applications as described in one or more of the preceding claims.
50. Any therapy that contains one or more methods as described in one or more of the preceding claims.
51. The use of any preparation according to one or more of the preceding claims, that includes the stimulation of stem cell-replication.
52. The use of any preparation according to one or more of the preceding claims, in combination with other signal proteins and peptides.
53. Any conceivable combination of two or more of the preceding claims, either resulting or not resulting in synergistic activity.

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CLAIMS:

-- 54. A method for quantitative structure function analysis research of biologically active proteins or peptides selected from human receptors such as interleukins, haemopoietic growth factors, peptide hormones or protein hormones, signal peptides or signal proteins, for the introduction of one or more of the following features: enhanced biological activity, enhanced stability, suppressed antigenicity, acquired antagonistic activity or cell inhibitory activity, said method comprising applying a specific chemical modification of selected amino acids using

- a) gradual chemical modification of the protein or peptide, followed by
b) monitoring the modification reaction with a mild and sensitive method such as non denaturing electrophoresis and/or electrospray mass spectrometry and said monitoring optionally further comprising confirming the overall structural integrity e.g. using Circular Dichroism Spectroscopy.
c) protease treatment,
d) mass spectrometry and
e) assaying biological activity of the modified product and optionally assaying stability of the modified product, said proteins or peptides preferably being selected from interleukins 1-8, interleukin 10, GM-CSF, TNF, insulin, prolactin and gamma IFN, more preferably GM-CSF, EPO or an interleukin being selected from interleukins 2-7 selected from the cytokine super family.

55. A method according to claim 1 wherein specific digestion with specific proteases and mass spectrometry is carried out for characterisation and localisation of the modified amino acids.

56. A method according to claim 1 or 2 wherein specific digestion with specific endoproteases and LDMS is carried out for characterisation and localisation of the modified amino acids, said endoprotease for example being Endo Glu C or Endo Lys C.

57. A method according to any of claims 1-3 wherein the modification is carried out by specific digestion with specific exoproteases and electrospray mass spectrometry is carried out for characterisation and localisation of the modified amino acids, suitably

the exoprotease is N terminal e.g. Cathepsine C or C terminal e.g. carboxypeptidase Y.

58. A method according to ^{claim 84 or 55} ~~any of claims 1-3~~, wherein the modification is chemical modification, said modification being alkylation and/or said modification being acylation, such as acetylation e.g. by Iodo acetate or succinylation e.g. by succinic anhydride, said modification suitably being a modification under gradually varying conditions, wherein one or more of the following conditions are varied as follows: pH between 5.0 and 7.0, preferably in steps of 0.5 pH units, and/or time or reagent-concentrations are varied.

59. A method according to claim ⁵⁸ ~~5~~, wherein the modification is carried out in the presence of phosphate buffer, preferably in combination with acetic anhydride.

60. A method according to one or more of the preceding claims, for the introduction of an antagonistic and/or cell inhibitory activity, wherein the modification has specificity to one or more residues that are involved in catalytic activity e.g. wherein the modification is within or in close proximity to a partial or complete catalytic center, said modification preferably changing the catalytic activity, suitably said residue is a histidine residue.

61. A method according to ^{claim 84 or 55} ~~any of the preceding claims~~, wherein the modification is within or in close proximity to a metal binding center, preferably a Zinc binding center, suitably said residue is a histidine residue.

62. A method according to ^{claim 84 or 55} ~~one or more of the preceding claims~~, wherein the modification is performed by reversibly denaturing the substrate and adding chelating agent to remove the metal ion e.g. in the presence of urea and EDTA, said urea preferably having a concentration larger than 5 M and said EDTA preferably having a concentration of 50 mM.

63. A method according to ^{claim 84 or 55} ~~one or more of the preceding claims~~, wherein the modification is specific for one type of amino acid, for instance an amine-residue and/or even is specific for only 1 amine-residue in the peptide or protein, said 1 amine for instance being

the N- terminus.

64. A method according to ^{claim 84 or 55} ~~any of the preceding claims~~ wherein the substrate is human interleukin-3, said method preferably providing interleukin 3 modified only at one or more of the following residues: Ala¹, His²⁶, Lys²⁸, Lys⁶⁶, His⁹⁵, His⁹⁸, Lys¹⁰⁰, or Lys¹¹⁶.

65. A method according to ^{claim 84 or 55} ~~any of the preceding claims~~ for the introduction of an antagonistic and/or cell inhibitory activity said method comprising disruption of phosphate binding.

66. A modified signal substance, preferably a protein hormone, peptide hormone, a growth factor, a hematopoietic growth factor, an interferon, an interleukin and/or a colony stimulating factor with an enhanced biological activity, antagonistic activity and/or cell inhibitory activity, wherein the modification is within or in close proximity to a partial or complete catalytic center, preferably such that the catalytic activity is changed, said modification further preferably being within or in close proximity to a metal binding center.

67. A modified signal substance being a Zinc binding signal peptide, preferably selected from Growth Hormone, prolactin and insulin, the same (cytokine) superfamily as the IL-3 receptor, preferably IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, Epo, IFN-gamma, more preferably selected from the following: IL-2, IL-3, IL-6, IFN-gamma, Growth Hormone, prolactin and insulin, said modified substance having an enhanced biological activity, antagonistic activity and/or cell inhibitory activity, wherein the modification is, preferably within or in close proximity to a Zinc binding center, such that the metal binding properties have been changed.

68. A substance according to claim ⁶⁷ ~~14~~, wherein the metal ion is within or in close proximity to a catalytic center, preferably said metal ion having a catalytic function in the unmodified substance.

69. A substance, as described in one or more of the preceding substance claims 13-15, wherein the modification for producing an antagonist is a chemical modification, preferably an alkylation, an acylation or molecular biological modification like a deletion mutation

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and/or a substitution mutation, most preferably the modification is an alkylation .

70. A substance, as described in one or more of the preceding substance claims 13-16, wherein the modification is of an amino acid involved in the binding of a metal ion, preferably a histidine residue.

71. A substance, as described in one or more of the preceding substance claims 13-17, wherein the affinity of the signal substance for the receptor has not decreased by more than a factor 10, preferably has remained the same and more preferably has increased.

72. A substance according to any of the preceding substance claims, 13-18 wherein the concentration of substance required for significant inhibition is suitable for clinical application i.e. less than a hundred fold higher than the native substance concentration, said substance optionally further having increased receptor binding capacity.

73. A substance according to any of the preceding substance claims, 13-19 wherein the substance is interleukin 3 preferably human interleukin 3, most preferably modified only at one or more of the following residues: Ala¹, His²⁶, Lys²⁸, Lys⁶⁶, His⁹⁵, His⁹⁸, Lys¹⁰⁰, or Lys¹¹⁶.

74. Substance according to claim ⁷³ 20, comprising at least one of the following characteristics

- 0.1 ng of the substance, modified IL-3 inhibits almost 50% of 3ng/ml native IL-3
- 3ng/ml of the substance, modified IL-3 suppresses 80-90% thymidine incorporation of 30-100 ng/ml control IL-3
- the substance modified IL-3 inhibits control IL-3 by a factor 10-100.

75. A substance, ^{according to one of claims 66-68} ~~as described in one or more of the preceding substance claims 13-21~~, wherein the substance has acquired one of the following combinations of characteristics

- a decreased stability and increased antagonistic activity for example acetylated IL-3,
- a decreased stability and increased agonistic activity e.g. N-terminally proteased IL-3 e.g. Cathepsin C treated IL-3,
- an increased stability and antagonistic activity e.g succinylated IL-3,

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- an increased stability in combination with an agonistic activity for example C-terminally proteased IL-3 e.g. Carboxypeptidase-Y treated IL-3.

76. Preparation for clinical application, containing a modified substance (both in mixed form and in chemically bound form) according to one or more of the preceding substance claims 13-22, optionally in combination with other signal proteins and peptides.

77. A method for preparing a substance according to ^{one of claims 66-68} ~~any of claims~~ 13-22 comprising carrying out the method steps as defined in ^{claim 84} ~~any of~~ claims 1-12.

78. A method of ^{obtaining at least inhibition or suppression} ~~inhibition, suppression and/or cure~~ of a HIV infection wherein the antibody levels are lowered by any of the following steps

- suppression of antibody production by B-cells, suppression of generation and/or maturation of B-cells, preferably said B cells being anti-HIV- antibody producing B-cells, preferably anti-HIV coat- antibody producing B-cells,
- plasmaphoresis, partial or complete plasma recovery or selective return of serum,
- *in vitro* removal of antibodies, preferably HIV-reactive antibodies, preferably HIV-envelope reactive antibodies,
- *in vivo* depletion, preferably with antibodies, preferably against HIV, preferably against the HIV envelope.
- leukaphoresis.

79. A method according to claim 25 comprising application of a preparation as described by claim 19 and/or application of a substance obtainable by a method according to any of the method claims 1-12.

80. A method according to claim ⁷⁸ ~~25 or 26~~, comprising application of bi-specific antibodies, preferably directed against the combination CD19/CD3 and or CD20/CD3.

81. A method according to ^{claims 78 and 80} ~~any of the preceding method claims 25-27~~, comprising application of B-cell apoptose inducing substances, preferably APO-1 and/or application of TGF- β as inhibitor of B-cell antibody production.

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82. A method for stimulating of stem cell-replication comprising application of a preparation according to claim ⁷²19 and/or a substance obtainable according to any of the method steps according to ^{claim 84}~~any of~~ ~~claims 1-12~~.

83. A method of gene therapy comprising applying a nucleic acid construct encoding a substance according to ^{one of} ~~claims 13-22~~ ⁶⁶⁻⁶⁸ to a subject to be treated, said therapy e.g. being directed at HIV infection. --

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